0273-2289/91/2829—0183$02.60

Kinetic Investigation and Mathematical Modeling of Methanogenesis of Glucose

S. V. KALYUZHNYY,*[,1] V. P. GACHOK,[2]
V. I. SKLYAR,[1] AND S. D. VARFOLOMEYEV[1]

[1]*Department of Chemical Enzymology, Chemistry Faculty, M. V. Lomonosov University, Moscow 119899, USSR; and*
[2]*Institute of Theoretical Physics, Ukranian Academy of Science, Kiev, USSR*

ABSTRACT

The kinetic regularities of anaerobic conversion of glucose, and intermediates of its decomposition (ethanol, butyrate, and acetate) by a microbial methanogenic association from anaerobic digester were investigated. Kinetic scheme for conversion of glucose is suggested, and the mathematical model based on the scheme is evolved.

The model includes growth and metabolism of three kinds of microorganisms—acid producents, and acetate- and hydrogen-utilizing methane producents; of cell lysis with consequent fermentation of "died biomass" to acetate, hydrogen, and carbon dioxide; of induction and repression of the enzyme responsible for decomposition of butyrate, and for a number of regulations depending on the concentrations of intermediates in glucose metabolism. The values of parameters of the model have been calculated, sufficiently describing the experimental regularities. The numerical experiments have enabled us to reveal and describe the principal regulating factors of glucose methanogenesis.

Index Entries: Kinetics; kinetic scheme; methanogenic consortium of microorganisms; mathematical model; parameters.

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

Although the problem of anaerobic fermentation has been studied for a long time, the kinetic regularities of the relevant processes and the participation of microbial interactions within them are still obscure. The growing interest in the kinetics of methanogenesis in recent years has resulted in an essential increase in constructing the mathematical models for the processes (*1–7*).

Mathematical simulation of anaerobic conversion of glucose, one of the key natural organic compounds, has been recently carried out (*2,7*). Oi et al. (*2*) suggested a simple model in which differentiation of microorganisms was neglected, and volatile fatty acids (VFA) were described by generalized concentrations. The model works satisfactorily for short time intervals, but in general, simplifies the understanding of real processes. In the previous work (*7*), we described the mathematical model of glucose methanogenesis by a specially constructed thermophilic association of microorganisms, known as "*Methanobacillus kuzneceovii.*" The model is differentiated on acid and methane producents, and includes a system of differential equations describing the concentrations of the main intermediates; it satisfies the kinetic regularities of the process during 250 h of operation. According to the model (*7*), however, the growth rates of microorganisms and their fermentation activities are strictly correlated, i.e., are described by the same equations; this is an evident shortcoming of the model.

A number of experimental facts that appeared on studying the conversion of glucose by consortium of microorganisms from effluents of anaerobic digesters are not described by the model previously developed for association of "*Methanobacillus kuzneceovii,*" the latter being significantly less rich in microbial composition. The aim of the present work is to study kinetically the methanogenesis of glucose in batch process, and to work out a more comprehensive mathematical model operative for more than 600 h, and able to reveal the kinetic mechanisms of anaerobic fermentation.

MATERIALS AND METHODS

Filtered effluent of anaerobic digester operating on cattle's manure, not adapted to the substrates, was used as a source of inoculate. Kinetic investigations (batch cultivation) were carried out at 35°C in hermetic 525 mL flasks, using nutrient medium previously described (*8*), in argon atmosphere without stirring. The vol of liquid phase was 200 mL (the vol of introduced inoculate was 10 mL), and the substrates for anerobic fermentation were added in concentration of 0.3–1.0 g/L.

Concentration of methane, hydrogen, and carbon dioxide in gaseous phase, and ethanol and VFA in liquid phase were determined by gas chromatographic method, as described previously (*8*). Solubility of hydrogen

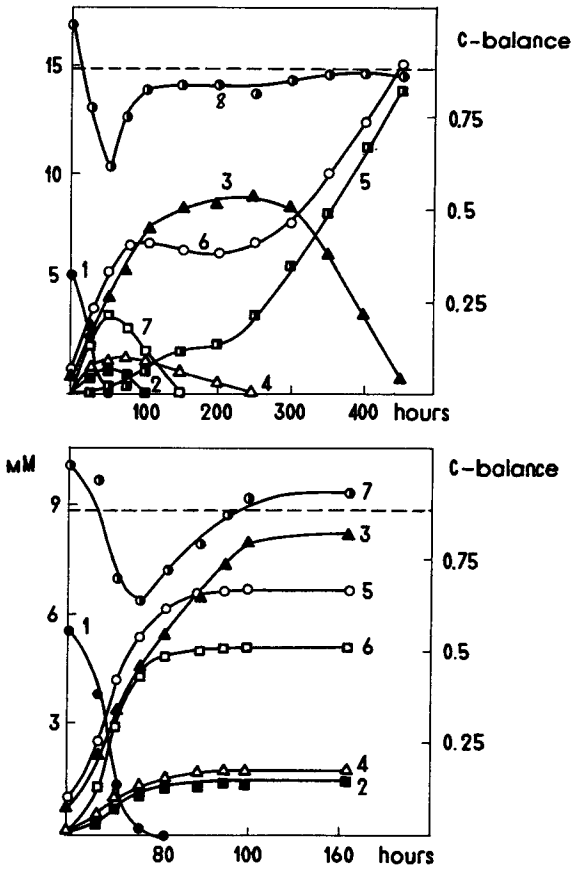

Fig. 1. Kinetics of anaerobic conversion of glucose (1 g/L) at the initial pH values equal to: a, 7.0; b, 5.0. a: 1, glucose; 2, ethanol; 3, acetate; 4, butyrate; 5, methane; 6, carbon dioxide; 7, hydrogen; 8, carbon balance. b: 1, glucose; 2, ethanol; 3, acetate; 4, butyrate; 5, carbon dioxide; 6, hydrogen; 7, carbon balance.

and methane in the medium was neglected. The overall content of CO_2 in reactor was calculated on the basis of its solubility in the medium, depending on temperature, pH, and pressure. The pressure in the reactor, increasing constantly in the course of reaction, was measured with manometer. Concentration of glucose was determined spectrophotometrically as also described previously (*8*). The average values of metabolite concentrations on figures below are obtained from four replicates.

RESULTS AND DISCUSSION

Kinetic Investigations

Kinetic studies of glucose biomethanogenesis show (*see* Fig. 1a) that hydrogen, carbon dioxide, ethanol, acetate, butyrate, and trace amounts of propionate are detected along with methane. The concentrations of

products of both the fermentation step (ethanol, butyrate, and hydrogen) and the acetogenic step (acetate and hydrogen) pass through maximums; hence, anaerobic fermentation is evidently a multistep process. After 50 h of fermentation, the carbon balance reaches the minimum. The dotted line in Fig. 1a corresponds to a 15% deficiency of carbon balance, which is a sum of 10% expense of organic compounds on the growth of microorganisms and of 5% error of experiment. The experimental fact that the deficiency in carbon balance exceeds a 15% limit only in the vicinity of 50 h means that we succeed in detecting practically all the principal intermediates in glucose biomethanogenesis. The minimum at 50 h probably results from the transient accumulation of oxyacids that cannot be detected by the gas chromatographic method used in the study.

It is known that acid- and methane-producing microorganisms have different pH optima. If anaerobic fermentation of glucose is studied at pH 5.5 (and not at pH 7.0 as described above), then acetogenic and methanogenic steps are eliminated and the only process that proceeds is an acid-producing step. The main products that are detected at the step are hydrogen, carbon dioxide, ethanol, acetate, and butyrate (*see* Fig 1b). It is also seen from the kinetic curve for the carbon balance that within the range 25–75 h, some undetected products are formed. Since propionate is present in trace amounts only, its formation will not be considered in subsequent mathematical model.

It follows from the data in Fig. 1b, that under the conditions of maximal accumulation of end product, the conversion of glucose (in molar concentrations) at the acid-producing step obeys the equation:

$$C_6H_{12}O_6 + 0.17H_2O \rightarrow 1.02H_2 + 1.14CO_2 + 0.29C_2H_5OH + \\ + 1.46CH_3COOH + 0.34C_3H_7COOH \quad (1)$$

During anaerobic fermentation of intermediates of glucose conversion, ethanol and butyrate are first transformed into acetate and hydrogen, followed by methanogenic step, Fig. 2. The following equations describe chemical conversion at the steps:

$$C_2H_5OH + H_2O \rightarrow CH_3COOH + 2H_2 \quad (2)$$

$$C_3H_7COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2 \quad (3)$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (4)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + H_2O \quad (5)$$

The thermodynamic calculations show that reactions (2,3) may proceed at partial pressure of hydrogen less than 0.15 and 0.002 atm, respectively, when concentrations of other substances are within the physiological range. Hence, permanent consumption of hydrogen and acetate by methanogenes, according to reactions (4,5), promotes shift of equilibrium to products at steps (2,3).

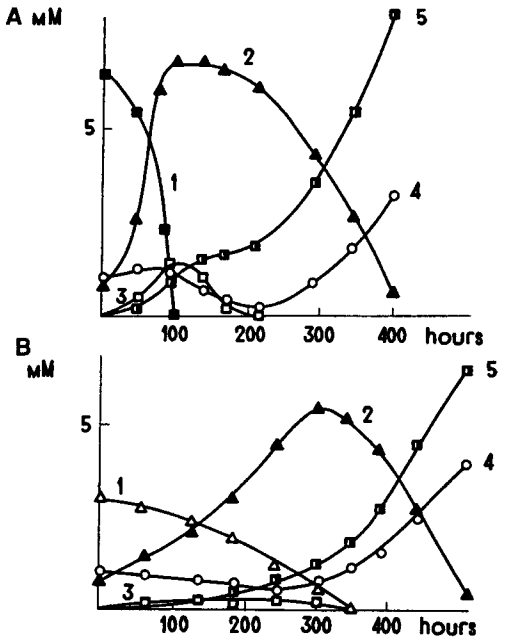

Fig. 2. Kinetics of anaerobic conversion of: a, ethanol (0.65 g/L); b, butyrate (0.3 g/L); initial pH value is equal to 7.0. a: 1, ethanol; 2, acetate; 3, hydrogen; 4, carbon dioxide; 5, methane. b: 1, butyrate; 2, acetate; 3, hydrogen; 4, carbon dioxide; 5, methane.

Thus, chemical scheme of methanogenesis of glucose (*1–5*) will be used for developing the kinetic scheme and mathematical models of the process. Additional experimental data for creation of a model were taken from the previous studies (*9,10*).

Description of Mathematical Model

The model was developed using the approach suggested in the previous studies (*7,11*). Analysis of experimental data shows that the model should consider at least three groups of microorganisms: X_1, X_2, and X_3. The group X_1 contains all acid producents and acetogenes; X_2, all acetate-utilizing methanogenes, and X_3, all hydrogen-utilizing methanogenes. Hence, X_1 brings about reactions (1–3), X_2, reaction (4), and X_3, reaction (5), and growth and fermentation processes are not interrelated. For simplicity, the groups of microorganisms X_1, X_2, and X_3, will be called cultures X_1, X_2, and X_3. The other assumption is that the model (at least at the present status) does not take into account the three-phasic character of the reaction medium and the influence on the biological systems of such physicochemical factors as pH, solubility of gases, and so on. These assumptions enable us to start with a simple model and further complicate it.

On substituting molar concentrations by weight concentrations (in g/L), the kinetic scheme of the model transforms to the following equations (where $S_1 = C_6H_{12}O_6$, $S_2 = C_2H_5OH$, $S_3 = CH_3COOH$, $S_4 = C_3H_7COOH$, $S_5 = CH_4$, $S_6 = CO_2$, $S_2 = H_2$):

$$S_1 \xrightarrow{w_1, X_1} 0.074S_2 + 0.487S_3 + 0.166S_4 + 0.278S_6 + 0.011S_7 \quad (6)$$

$$S_1 \xrightarrow{W_1, X_1} X_1 \quad (7)$$

$$S_2 \xrightarrow{w_2, X_1} 1.304S_3 + 0.087S_7 \quad (8)$$

$$S_2 \xrightarrow{W_2, X_1} X_1 \quad (9)$$

$$S_4 \xrightarrow{w_4, X_1} 1.364S_3 + 0.045S_7 \quad (10)$$

$$S_4 \xrightarrow{W_4, X_1} X_1 \quad (11)$$

$$S_3 \xrightarrow{w_3, X_2} 0.267S_5 + 0.733S_6 \quad (12)$$

$$S_3 \xrightarrow{W_3, X_2} X_2 \quad (13)$$

$$S_6 + 0.182S_7 \xrightarrow{w_5, X_3} 0.3674_5 \quad (14)$$

$$S_6 + 0.091S_7 \xrightarrow{W_5, X_3} X_3 \quad (15)$$

According to the scheme and to our assumption, the growth of culture X_1 on substrates S_1, S_2, and S_3 proceeds independently and simultaneously, and is described by the following equation:

$$dX_1/dt = m_1W_1 + 1.304m_2W_2 + 1.364m_4W_4 - a_1X_1 \quad (16)$$

where the rates of growth on each substrate are described by the functions:

$$W_1 = S_1X_1/(L_1 + S_1 + L_2X_1 + nS_7) \quad (17)$$

$$W_2 = S_2X_1M_6/((L_3 + S_2 + L_4X_1 + M_5S_7)(M_6 + S_2)) \quad (18)$$

$$W_4 = S_4X_1M_{10}/((L_8 + S_4 + L_9X_1 + K_2S_7)(M_{10} + S_4)) \quad (19)$$

Description of the growth rate of the culture is based on the classical Monod equation, complicated by self-inhibition of cells when their concentration is high, and by inhibition of their growth by hydrogen. Besides, Eqs. (18, 19) account additionally for inhibition by high concentrations of ethanol and butyrate, respectively.

Since we consider a wide temporal interval (600 h and more), cell lysis is displayed in kinetics of substrate transformation. The model takes this into account by introduction into Eq. (16) of the additional negative term with parameter a_1.

Fermentation activity of culture X_1 consists in catalyzing the reactions (6, 8, 10). Hence, the rates of the processes are described by the following equations:

$$w_1 = V(X_1)S_1/(K_1 + S_1 + M_1(S_2 + S_3 + S_4 + S_7)) \quad (20)$$

$$w_2 = V(X_1)S_2/K_8 + S_2 + M_2S_3 + M_9S_7) \quad (21)$$

$$w_4 = V(E)S_4/(K_9 + S_4 + M_4S_3 + M_7S_7) \quad (22)$$

and further on, $V(Y) = Y/(K_{10} + Y)$. The rate of glucose conversion is proportional to fermentation activity of the culture X_1, in turn determined by classical Michaelis equation, with inhibition by products of reaction (6). Factor $V(X_1)$ accounts that only a fraction of cells X_1 possesses fermentation activity, and its value changes constantly during the cell growth. The rate of ethanol transformation is similarly determined by considering the inhibition by acetate and hydrogen.

Finally, the rate of butyrate transformation depends on biosynthesis of enzymatic systems (enzyme E in the model), and is also inhibited by acetate and hydrogen. Thus, a group of microorganisms, so-called obligate proton reducers, that transform butyrate into acetate and hydrogen are presented in the model by the induced biosynthesis of enzyme E by culture X_1, according to the following equation:

$$dE/dt = E_0V(X_1)S_4^2N_2/((b + S_4^2)(N_2 + S_7 + N_1S_4)) - a_4E \quad (23)$$

where E_0 is the rate of biosynthesis of enzyme E; b is a parameter regulating the induction by butyrate; N_1 and N_2 are parameters responsible for repression of biosynthesis by butyrate and hydrogen; a_4 is a parameter of inactivation of enzyme E.

Thus, the growth of culture X_1 on glucose and fermentation of the latter determines the acidogenic step. The kinetic equation for glucose in this case appears as:

$$dS_1/dt = -l_1w_1 - m_1W_1 \quad (24)$$

In turn, the acetogenic step is also determined by the growth of culture X_1 on ethanol and butyrate and their transformation into acetate and hydrogen. The corresponding kinetic equations for ethanol and butyrate are the following:

$$dS_2dt = 0.074w_1 - l_2w_2 - m_2W_2 \quad (25)$$

$$dS_4dt = 0.166w_1 - l_4w_4 - m_4W_4 \quad (26)$$

Acetate, carbon dioxide, and hydrogen accumulated at acid- and acetate-producing steps are further consumed by growing methanogenic cultures X_2 and X_3; the processes are described by the following kinetic equations:

$$dX_2/dt = m_3W_3 - a_2X_2 \quad (27)$$

$$dX_3/dt = 0.682m_5W_5 - a_3X_3 \quad (28)$$

where the growth rates of cultures X_2 and X_3 appear in the forms:

$$W_3 = S_3X_2M_{11}/((L_5 + S_3 + L_6X_2 + L_7S_4 + M_8S_7)(M_{11} + S_3 + M_{12}S_2)) \quad (29)$$

$$W_5 = S_6S_7X_3/(L_{10} + L_{11}S_6 + L_{12}X_3 + L_{13}S_7 + L_{14}S_6S_7) \quad (30)$$

The growth of culture X_2 is inhibited by high concentrations of butyrate, hydrogen, ethanol, acetate, and X_2. The growth of culture X_3 depends on concentrations of two substrates and involves only mutual inhibition of cells. Negative terms in Eqs. (27, 28) take into account lysis of cells.

The rates of fermentation reactions carried out by cultures X_2 and X_3 are described by the following equations:

$$w_3 = V(X_2)S_3/(K_3 + S_3 + M_3S_7) \quad (31)$$

$$w_5 = V(X_3)S_6S_7/(K_5 + K_6S_6 + K_7S_7) \quad (32)$$

The rate of conversion of acetate into methane is inhibited by hydrogen, and we assume that utilization of hydrogen and carbon dioxide proceeds without any complications.

The equations for methanogenic substrates appear in the forms:

$$dS_3/dt = 0.487w_1 + 1.3041_2w_2 - l_3w_3 - m_3W_3 + 1.364l_4w_4 + a_5(a_1X_1 + a_2X_2 + a_3X_3) \quad (33)$$

$$dS_6/dt = 0.278w_1 + 0.733l_3w_3 - l_5w_5 - m_5W_5 + a_6(a_1X_1 + a_2X_2 + a_3X_3) \quad (34)$$

$$dS_7/dt = 0.011w_1 + 0.045(l_4w_4 + m_4W_4) + 0.087(l_2w_2 + m_2W_2) - 0.182l_5w_5 - 0.091m_5W_5 + a_7(a_1X_1 + a_2X_2 + a_3X_3) \quad (35)$$

The last item in each of the Eqs. (33–35) determines the increase in concentrations of acetate, carbon dioxide, and hydrogen at the expense of cell lysis. We assume that destruction of biomass finally results in formation of only the latter three substances, according to the following equation:

$$\underset{\text{biomass}}{(CH_2O)} + H_2O \rightarrow a_5CH_3COOH + a_6CO_2 + a_7H_2 \quad (36)$$

Finally, kinetic equation for methane production appears in the form:

$$dS_5/dt = 0.267l_3w_3 + 0.364l_5w_5 \quad (37)$$

Further, the system of Eqs. (16–35, 37) will be used for describing the biomethanogenesis of glucose.

Application of the Model

Verification of the model and introduction of new regulating factors was done in parallel with numerical experiments. The values of parameters were consequently selected on modeling of different initial states of the biosystem in order that kinetics of the simulated process agrees with the

temporal requirements of the experiment, and describes qualitatively its characteristic features (literary data were taken as first approximations of the parameters). On selecting the specific growth rates and metabolic coefficients, we tried to obtain the values of growth yields not differing greatly from 10% that corresponds to the literary data on accumulation of biomass during methanogenesis (*12*).

The values of parameters satisfying to the model of glucose methanogenesis developed by us are listed below; dimensions of the parameters are combinations of concentrations (in g/L) and time (in h):

$$\begin{aligned} &l_1 = 0.5;\ l_2 = 0.1;\ l_3 = 0.035;\ l_4 = 0.015;\ l_5 = 0.1;\ K_1 = 0.1;\ K_2 = 1;\\ &K_3 = 0.14;\ K_4 = 0.8;\ K_5 = 0.015;\ K_6 = 0.08;\ K_7 = 0.03;\ K_8 = K_9 =\\ &K_{10} = 0.1;\ N_1 = 0.1;\ N_2 = 0.009;\ b = 0.02;\ n = 0.1;\ M_1 = 3;\ M_2 =\\ &M_3 = 0.7;\ M_4 = 0.3;\ M_5 = 1;\ M_6 = 0.07;\ M_7 = 10;\ M_8 = 1;\ M_9 = 1;\\ &M_{10} = 0.03;\ M_{11} = 0.07;\ M_{12} = 1;\ m_1 = 0.012;\ m_2 = m_4 = 0.02;\ m_3 = \\ &0.04;\ m_5 = 0.1;\ a_1 = a_2 = a_3 = 0.01;\ a_4 = 0.04;\ L_1 = L_3 = L_8 =\\ &0.024;\ L_2 = L_9 = 0.4;\ L_4 = 0.5;\ L_5 = 0.05;\ L_6 = L_{14} = 0.1;\ L_7 = 0.7;\\ &L_{10} = 0.002;\ L_{11} = 0.01;\ L_{12} = 0.03;\ L_{13} = 0.02. \end{aligned} \tag{38}$$

Coefficients for Eq. (36) were chosen in assumption that 60% of the lysed biomass transforms into acetate, i.e., $a_5 = 0.6$. Other coefficients are obtained unambiguously from Eq. (37), taking into account the conditions of electron balance: $a_6 = 0.59$, $a_7 = 0.04$.

Let us fix the initial state of bioreactor when it contains only the inoculate and substances introduced with it (all in g/L):

$$\begin{aligned} &S_1^0 = S_2^0 = S_4^0 = S_5^0 = 0;\ S_3^0 = S_6^0 = 0.05;\ S_7^0 = 0.001;\\ &X_1^0 = X_2^0 = X_3^0 = 0.003;\ E^0 = 0.001 \end{aligned} \tag{39}$$

Let us consider three most character kinetic situations for methanogenesis, starting from different initial states. Taking the concentration of glucose as initial state (39), $S_1^0 = 1$ g/L, it can be seen from Fig. 3a that anaerobic fermentation proceeds through a number of steps. Formation of methane is completed within 450 h, the acidogenic step proceeds during 80 h and acetogenic proceeds through 300 h, that corresponds well to experimental data, Fig. 1a. Appearance of butyrate and hydrogen in the system leads to inhibition of growth of the culture X_2, and their utilization promotes acetoclastic methanogenesis that is perfectly seen from kinetic curve of acetate accumulation. When the process was completed, 11% of the substrates for the growth of the culture X_1 ($S_1 + S_2 + S_3$), 9% of the substrate for the culture X_2 (S_3), and 8% of the substrate for the culture X_3 ($S_6 + S_7$) were consumed, which corresponds well to the literary data (*12*).

Fig. 3b shows numerical results for development of methanogenesis starting from the initial state (39), when $S_2^0 = 0.65$ g/L. Formation of methane at the first steps needs the participation of the culture X_3, decreasing the concentration of hydrogen in the system, and thereby promoting the growth of the culture X_2. The process of methanogenesis is completed

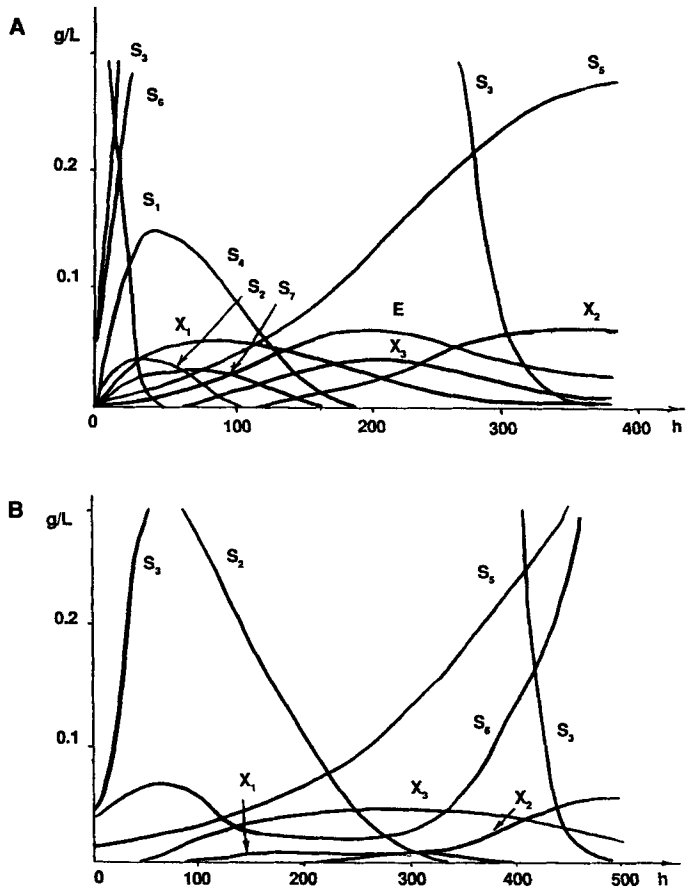

Fig. 3. Results of modeling the kinetics of anaerobic conversion of: a, glucose (1 g/L); b, ethanol (0.65 g/L).

within 400 h. Anaerobic fermentation of butyrate from the initial state (39) (when $S_4^0 = 0.3$ g/L) is characterized by special features, the principal being the inhibition of acetoclastic methanogene (Fig. 4). The process is determined by the growth of the culture X_1 on butyrate with subsequent biosynthesis of enzyme E. After decreasing the concentration of butyrate, production of methane from acetate is activated and is completed within 600 h. The results of simulation shown in Figs. 3b and 4 also correspond to the experimental data (Fig. 2).

Let us fix the accepted values of parameters (38) together with the initial state (39) and consider them as a starting system. Let us illustrate briefly how the principal regulating factors of methanogenesis can be revealed and described, based on the model.

Role of the Culture X_1

On varying the initial concentration of glucose from 0.25 to 1.25 g/L, the time of completion of the acidogenic step remains practically constant

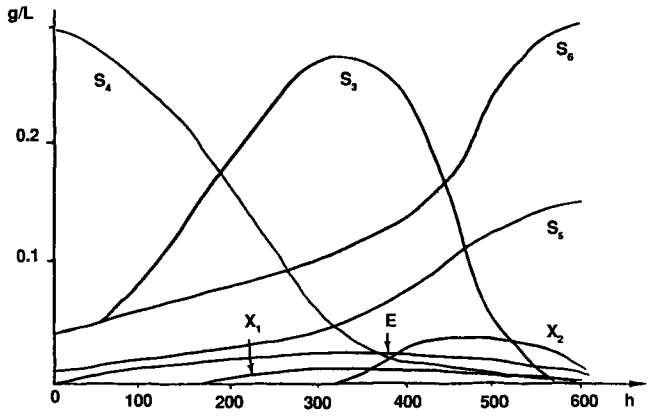

Fig. 4. Results of modeling the kinetics of anaerobic conversion of butyrate (0.3 g/L).

and equal to 45 h. Similar situation is also characteristic of acetogenic step, since the latter is also carried out by culture X_1. In all variants, the time needed for completion of this step, i.e., for complete disappearance of the most hardly converted substrate, butyrate, is approximately equal to 180 h. The methanogenic step is partially overlapped with the acetogenic step, and its active development starts after butyrate is exhausted in the system.

On varying the parameter m_1 that determines the growth rate of culture X_1 on glucose, the time for its growth at the acidogenic step does not depend on m_1, and is equal to 45 h. A small increase in X_1 during the interval of 45–180 h is a result of the acetogenic growth of the culture on ethanol and butyrate. The characteristic feature of the process is that the maximal activity of enzyme E responsible for the rate of butyrate fermentation is reached at 150 h, or in other words, a lag period in biosynthesis of enzyme E is observed.

The parameter m_4 that determines the growth rate of culture X_1 on butyrate has a great influence on its growth in the absence of glucose, and on conversion of butyrate into biomass X_1, Fig. 5A. Increase in concentration of X_1 leads to substantial increase in concentration of enzyme E in cells which, in turn, results in a more rapid conversion of butyrate. If this parameter is varied for the initial state where only glucose is in the system, then influence of m_4 is displayed both on butyrate utilization and on kinetics of methanogenic step.

Role of the Culture X_2

The abovementioned high sensitivity of kinetics of the growth of the culture X_2, and of its fermentation activity in response to the presence of butyrate, may be illustrated in numerical experiment with the help of parameter L_7; by its changing in the product L_7S_4, we model response of the system for a change in butyrate levels, *see* Fig. 5b. Since the growth of the culture

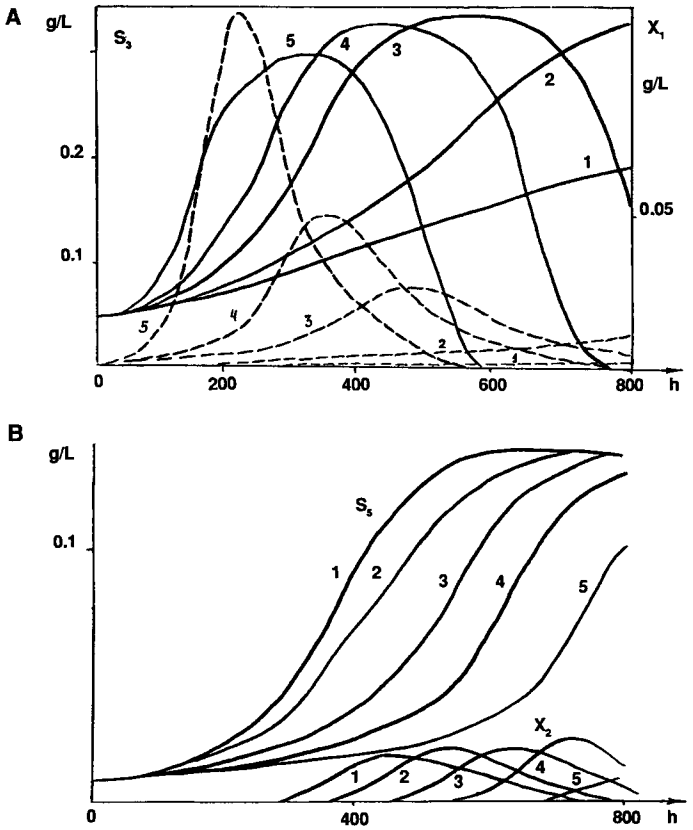

Fig. 5a. Data of numerical experiment on kinetics of growth of the culture X_1 (---) and accumulation of acetate (——) on varying the parameter m_4: 1, 0.014; 2, 0.02; 3, 0.03; 4, 0.04; 5, 0.07 (S_4^0=0.3 g/L).

b. Data of numerical experiment on kinetics of growth of the culture X_2 and accumulation of methane on varying the parameter L_7: 1, 0.5; 2, 0.7; 3, 1.5; 4, 2.5; 5, 5 (S_4^0=0.3 g/L).

X_2 decelerates on increase in L_7, a lag period for methane formation at the acetoclastic step is observed. Parameter L_7 determines the time for the acidogenic step, and for the beginning of the methanogenic step at different initial conditions. Thus, comparing the times with experimental values, we find the proper value, which is stated in the list (39). Similarly, a choice of other parameters of the suggested model is made, and relations between them are established.

Along with butyrate, hydrogen may also inhibit the growth of the culture X_2; this is shown by numerical experiment on change of parameter M_8. Increase in hydrogen concentrations results in prolongation of a lag period for methanogenic step.

Role of the Culture X_3

The culture X_3 is the terminal in the simulated association of the cultures. The influence of culture X_3 on the other cultures depends on whether

this is the only consument of hydrogen, i.e., the most important regulator in the whole biosystem. Evidently, the intensive growth of the culture X_3 decreases greatly the level of hydrogen, and in this way, influences the growth of culture X_2 and biosynthesis of enzyme E.

CONCLUSION

In conclusion, it should be mentioned that the model describes adequately the experimental data, and can be used for a more thorough study of regularities of methanogenesis. Further improvement of the model may be based on consideration of the role of growth and metabolism of bacteria responsible for utilization of butyrate and other VFA, and of the three-phasic character of the reaction system, pH-dependencies, and induction of enzymes of cellulase complex in case of methanogenesis of cellulose. Other applications of the model and its development will be described in further publications.

REFERENCES

1. James, A. (eds.) (1981), *Mathematical Model for Control of Water Pollution,* Mir Press, Moscow, pp. 321–346.
2. Oi, S., Tamura, S., Nukina, Y., Tanaka, T., and Taniguchi, M. (1984), *Agric. Biol. Chem.* **48,** 1329.
3. Wang, Y.-T., Suidan, M. T. (1987), *Biotechnol. Bioeng.* **30,** 158.
4. Yang, S. T., Okos, M. R. (1987), *Biotechnol. Bioeng.* **30,** 661.
5. Beba, A., Atalay, F. S. (1987), *Biomass* **11,** 173.
6. Young, A. (1989), *J. Chem. Tech. Biotechnol.* **46,** 189.
7. Varfolomeyev, S. D., Kalyuzhnyy, S. V., and Spivak, S. I. (1989), *Appl. Biochem. Biotechnol.* **22,** 351.
8. Varfolomeyev, S. D. and Kalyuzhnyy, S. V. (1989), *Appl. Biochem. Biotechnol.* **22,** 331.
9. Sklyar, V. I., Kalyuzhnyy, S. V., Mihantéva, T. V., Kovaley, G. V., Sinitsyn, A. P., and Varfolomeyev, S. D. (1987), *Bioteckhnologiya* **3,** 87.
10. Kalyuzhnyy, S. V., Davlyatshina, M. A., and Varfolomeyev, S. D. (1989), *Doklady AN USSR* **309,** 244.
11. Gachok, V. P. (1988), *Kinetics of Biochemical Processes,* Naukova Dumka Press, Kiev, p. 220.
12. Kalyuzhnyy, S. V., Puzankov, A. G., and Varfolomeyev, S. D. (1988), *Biogas: Problems and Solutions,* VINITI Press, Moscow, p. 177.